



# Functional Contributions of Carbohydrate on AIDS Virus Glycoprotein

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## REVIEW

# Functional Contributions of Carbohydrate on AIDS Virus Glycoprotein

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Envelope glycoprotein spikes on the surface of the human immunodeficiency virus (HIV†) are used by the virus to bind to cellular receptors to gain entry into target cells. As such, the envelope spikes are the targets of antibodies that can neutralize viral infectivity. Fifty percent or more of the mass of the viral-encoded surface glycoprotein of HIV, and of its close monkey relative simian immunodeficiency virus (SIV), is actually carbohydrate; it is one of the most heavily glycosylated proteins that can be found in mammals. It has been clearly demonstrated that one of the functions of this carbohydrate is to shield viral epitopes that would otherwise be the direct target of antibodies that could neutralize viral infection. In addition, it is now generally accepted that the carbohydrate on the viral envelope glycoprotein is recognized by multiple cellular lectins of the host lymphoreticular system, and these interactions play a role in the dissemination of virus within the host as well as the release of modulatory cytokines. Our work recently demonstrated fundamental differences in the composition of the carbohydrate on HIV type 1, the cause of the AIDS pandemic, versus the SIV in the sooty mangabey monkey, a natural host that does not develop disease from its infection. We now speculate that this fundamental difference in carbohydrate composition reflects evolutionary pressures on both virus and host. Furthermore, carbohydrate composition on the virus and genetic differences in carbohydrate-sensing proteins of the host could be critically important for the generalized lymphoid activation that characterizes the acquired immunodeficiency syndrome (AIDS).

## INTRODUCTION

Incorporation of the envelope protein (Env) spike is essential for the infectivity of HIV and SIV. Env is synthesized from a singly spliced viral mRNA and directed to the secretory pathway of the infected cell by an amino terminal signal peptide of 25 amino

acids [1]. The Env precursor protein, gp160, oligomerizes into trimers through interactions of the transmembrane protein domain [2,3,4]. Then, cellular furin or furin-like proteases cleave the oligomerized gp160s into the surface subunit (gp120) and the transmembrane protein (gp41), which are nonco-

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†Abbreviations: HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; Env, envelope protein; GalNAc, N-acetyl-galactosamine; Ser, serine; Thr, threonine; SIVmac239, SIV from rhesus macaque; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; CV-N, Cyanovirin-N; MBL, mannose binding lectin.

valently associated in the Env protein complex [5,6]. In most cell types, Env is trafficked to the plasma membrane, where it is incorporated into virus particles [7]. For macrophage cells, Env is incorporated into virus particles largely at the multi-vesicular body [8]. Virus is contained in the macrophage multivesicular body prior to the fusion of this compartment with the plasma membrane when infectious virions are released [8].

The Envs of HIV and SIV are heavily modified with carbohydrate. The attachment of N-linked carbohydrate is initiated when the carbohydrate core oligosaccharide (two N-acetylglucosamine, nine mannose, and three glucose) is transferred *en bloc* to the asparagine of the N-linked consensus sequence N-X-S or N-X-T, where X is any amino acid except a proline [9-13]. Then, the glucose is removed to form high-mannose carbohydrate chains that terminate in mannose [12]. High-mannose carbohydrate may be further processed into complex or hybrid oligosaccharides [14]. Fully processed complex carbohydrate chains terminate in galactose, N-acetylglucosamine, sialic acid, or glucose [15,16]. Hybrid carbohydrate chains have one branch that terminates in mannose and another branch that terminates in a sugar of the complex type [17]. Therefore, at each occupied site, the N-linked carbohydrate chain may be one of three types: high-mannose, complex, or hybrid.

In addition to the attachment of N-linked carbohydrate, the Envs of HIV and SIV also may be modified with O-linked carbohydrate in the secretory pathway of the infected cell. This type of carbohydrate attachment, commonly referred to as mucin-type [18], initiates with the covalent attachment of N-acetylgalactosamine (GalNAc) to the hydroxyl group of serine (Ser) and/or threonine (Thr) to form the Tn antigen [19,20]. There are no clear-cut rules that distinguish a glycosylated Ser or Thr from a non-glycosylated Ser or Thr in the primary protein sequence [18]. After the addition of GalNAc, the carbohydrate chain may then be elongated by the addition of galactose, N-acetylglucosamine, and sialic acid in different combinations and linkages [18,21]. The

Tn antigen, core 1, immature core 2, core 2, and the sialylated versions are the most common mucin-type O-linked carbohydrate [22].

### **FUNCTION OF N-LINKED CARBOHYDRATE IN FORMATION OF A FUSION-COMPETENT ENV PROTEIN COMPLEX**

Initial observations of N-linked carbohydrate contributing to the function of Env were made in studies where virus made in the presence of glucosidase inhibitors displayed impaired infectivity compared to virus made in the absence of inhibitors [23,24]. The inhibition of infectivity or syncytium formation could be attributed to an altered N-linked glycosylation pattern of Env, a decreased cell surface expression of the mature Env glycoprotein, and a decreased processing of the precursor gp160 into gp120 and gp41 compared to that of Env from mock treated cells [25].

The effects of carbohydrate on folding, processing, and efficient intracellular transport of Env also were detected for viral variants lacking a subset of N-linked glycans from either gp120 or gp41 [26-29]. Li et al. identified a subset of N-linked carbohydrate that was important for proper folding of the CD4 binding pocket of gp120 [27]. Reitter et al. and Pikora et al. noted a decreased processing from "gp160" to "gp120" for mutants that lack three sites in combination within gp120 [28,29]. Fenouillet and Jones reported that a mutant precursor of Env that lacked three conserved N-linked sites of HIV type 1 (HIV-1) gp41 was deficient for transport resulting in a deficient processing of gp160 [26].

The role of N-linked carbohydrate is thus particularly important for the assembly of a fusion-competent Env spike. After the Env spike is assembled, enzymatic removal of N-linked glycosylation does not affect the functional conformation [30-35].

### **FUNCTION OF CARBOHYDRATE IN SHIELDING EPITOPES**

In a seminal paper, variants of pathogenic SIV from rhesus macaque (SIV-

mac239) that lacked two of 23 N-linked glycosylation sites of gp120 were much better at eliciting neutralizing antibodies and much more sensitive targets for neutralization than the parental SIVmac239 strain [36]. A variant of SIVmac239 that lacked five N-linked sites in gp120 replicated normally in monkeys for the first two weeks [36]. Notably, the infection was then controlled, at least in part by neutralizing antibodies, at low or undetectable levels for more than two years [36]. This study indicated that removal of carbohydrate triggered an antibody response that effectively controlled viral replication and also provided evidence for the necessity of an intact glycan shield to protect the virus against the attack of the host immune system.

The importance of an intact carbohydrate shield also was highlighted for HIV-1 in a report in which the glycosylation pattern of viral escape variants was introduced into two sensitive strains [37]. The addition of multiple N-linked sites into both sensitive strains was sufficient to acquire a neutralization-resistant phenotype, thereby providing evidence of a continuously evolving glycan shield in HIV-1 [37].

Now, it is generally accepted that carbohydrate covers portions of antigenic sites of the Env protein such that increased numbers of N-linked glycosylation sites in gp120 of HIV-1 and SIV are associated with an increased resistance to antibody-mediated neutralization, while loss of N-linked sites frequently results in virus that is more susceptible to antibody-mediated neutralization [36,38-48]. Consistent with N-linked carbohydrate physically hindering antibody recognition of epitopes that would otherwise be the direct targets of neutralizing antibodies, variants of SIVmac239 that lacked N-linked sites in gp120 or gp41 elicited antibodies with new specificities when compared to antibodies from SIVmac239 sera [49,50].

What about O-linked carbohydrate and the carbohydrate shield? Chackerian et al. beautifully demonstrated a role for O-linked carbohydrate in shielding SIV from neutralization by host antibodies [39]. Similarly, a rhesus macaque that developed a neutraliz-

ing antibody response to SIVmac239 had an escape variant that introduced a Thr into an existing O-linked carbohydrate attachment site [46,51].

## **HIV-1 GP120 CARBOHYDRATE-HOST LECTIN INTERACTIONS**

The carbohydrate of HIV-1 and SIV gp120 has been found to bind lectins on the surface of dendritic cells, enhance the infection of HIV permissive cells, and modulate the immune response. Geijtenbeek et al. elegantly showed that binding of HIV-1 gp120 to dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), a type II transmembrane C-type lectin with a single C-terminal carbohydrate recognition domain [52], facilitated the infection of HIV permissive T-cells via a trans-infection pathway [53]. Virions bound to macrophage cells expressing DC-SIGN could trans-infect CD4<sup>+</sup> T-cells four days after capture, and this trans-infection could be blocked by an antibody to DC-SIGN [53]. DC-SIGN binds to HIV-1 gp120 via carbohydrate since the removal of specific glycans from gp120 decreased the capacity of DC-SIGN to bind, which led to a decreased efficiency of trans-infection of CD4<sup>+</sup> T-cells [54].

HIV-1 gp120 carbohydrate functions through binding DC-SIGN to activate crucial components of the immune system. Binding of HIV-1 gp120 to DC-SIGN induced Raf-1 dependent phosphorylation of the NF- $\kappa$ B subunit p65 at Ser276 that recruited transcription-elongation factor pTEF-b to nascent transcripts for the synthesis of full-length viral transcripts leading to a productive infection of dendritic cells [55]. Separately, binding of the high-mannose carbohydrate of gp120 to DC-SIGN also induced signaling of the ERK pathway in dendritic cells that resulted in the release of IL-10, an immunosuppressive cytokine that may contribute to the T<sub>H</sub>2 bias of the anti-gp120 immune response [56,57,58].

Carbohydrate of HIV-1 binds to many C-type lectins as well as other lectins of the human immune system [59,60,61]. Another

**Table 1.** Description of the viral host, carbohydrate composition, and disease progression.

Virus	Host	N-linked High-mannose carbohydrate	O-linked carbohydrate <sup>a</sup>	Progression to disease
HIV-1 <sup>b</sup>	human	high	no	yes
SIVcpz <sup>b</sup>	chimpanzee	high	no	+/-
SIVsm <sup>c</sup>	sooty mangabey	low	yes	no
SIVmac <sup>c</sup>	rhesus macaque	low	yes	yes
HIV-2 <sup>c</sup>	human	low	yes	slow

a. of the non- and mono-sialylated core 1 types.

b. Cross-species transmission of SIVcpz to humans is believed to be the source of HIV-1.

c. Cross-species transmission of SIVsm to rhesus macaques and to humans is thought to be the source of SIVmac and HIV-2, respectively.

noteworthy binding partner of the carbohydrate on gp120 is the soluble mannose binding lectin (MBL) [62]. MBL is a member of the collagenous lectin (collectin) family of proteins, large multimeric proteins that consist of collagen and a Ca<sup>2+</sup>-dependent carbohydrate-binding domain [63]. The collectins are a part of the larger group of C-type lectins [63]. *In vitro*, the mannose binding lectin inhibits trans-infection mediated by macrophage cells expressing DC-SIGN [64]. *In vivo*, the level of MBL in human sera varies due to polymorphisms in the promoter of the gene and point mutations within the protein-coding region [65]. In some studies, low levels of MBL have been associated with an increased susceptibility to HIV-1 infection and an increased progression of disease, supporting the theory that either high or medium levels of MBL are required for the control of disease progression [65].

### HIV-1 GP120 CARBOHYDRATE AND THERAPEUTIC LECTINS

The lectin Cyanovirin-N (CV-N), an 11 Kda protein isolated from blue green algae, directly targets carbohydrates on the Env of HIV to block viral entry into cells [66]. In 2003, CV-N was applied in a gel to the rectum of monkeys to effectively protect against infection of SHIV89.6, a virus that is primarily SIV but expresses the Env of HIV [67]. Subsequent to this proof of principle experiment, interest in the use of CV-N in human trials waned due to safety concerns. Suboptimal concentrations of CV-

N enhanced HIV infection of human peripheral blood mononuclear cells [68]. Also, the process of cell division was stimulated in the presence of CV-N [69]. Recently, a similar lectin has been identified that reportedly does not have these negative side effects [70], which may prove to be a therapeutic directed toward the carbohydrate of HIV-1 Env.

### CARBOHYDRATE COMPOSITION OF THE GP120S FROM HIV-1 AND SIV FROM SOOTY MANGABEYS (SIVSM)

Recently, we have identified fundamental differences in both the N-linked and O-linked carbohydrate composition for HIV-1 versus SIVsm [51,71]. We defined two N-linked sites in the external surface glycoprotein gp120 and one in the gp41 transmembrane glycoprotein whose mutation imparted high-level resistance to the inhibitory effects of the high-mannose carbohydrate binding lectins GNA and HHA onto cloned SIVmac239 [71]. This was in contrast to selection of HIV-1, where the GNA and HHA-resistant population lacked eight of 24 N-linked sites in gp120 [72]. We used a GNA-binding ELISA to show that assorted HIV-1 and SIVcpz gp120s are consistently and considerably higher in high-mannose composition than assorted gp120s from SIVmac, SIVsm, and HIV type 2 (HIV-2) (Table 1) [71]. In a separate study, we identified an O-linked carbohydrate attachment region in the gp120 of SIVmac239, whose mutation

imparted high-level resistance to the inhibitory effects of jacalin, a lectin that binds O-linked carbohydrate, onto cloned SIVmac239 [51]. Modification of the gp120 of SIVmac239 with non- and mono-sialylated core 1 carbohydrate was confirmed by mass spectrometry [51]. Furthermore, using a jacalin-binding ELISA, we showed that assorted gp120s from SIVmac and SIVsm were consistently modified with non- and mono-sialylated core 1 mucin-type O-linked carbohydrate while assorted HIV-1 and SIVcpz gp120s consistently lacked these jacalin-sensitive structures (Table 1) [51].

### **FUNDAMENTAL DIFFERENCES OF CARBOHYDRATE COMPOSITION BETWEEN HIV-1 AND SIVSM AND INTERACTIONS WITH LECTINS OF THE HOST LYMPHORETICULAR SYSTEM**

The best characterized interaction of the carbohydrate of HIV and SIV Env is the binding to the host lectin DC-SIGN. DC-SIGN on dendritic cells has been shown to mediate trans-infection of permissive CD4<sup>+</sup> T-cells for HIV-1, HIV-2, SIVmac, SIVmne, and SIV from African green monkeys [73,74,75]. In trans-infection experiments, HIV-1 Env that had a high high-mannose carbohydrate composition mediated the infection of CD4<sup>+</sup> T-cells seven times more than that of the low high-mannose HIV-2 Env [74].

Interestingly, even though the DC-SIGN of multiple species of monkeys have been shown to mediate the trans-infection of virus, no animal experiment has been reported showing the contribution of trans-infection mediated by DC-SIGN to the dissemination of virus in the host. This is probably due to the multitude of lectins that can mediate trans-infection. In rhesus macaques, dendritic cells have been reported to efficiently transmit primate lentiviruses independently of DC-SIGN [76].

### **CONCLUDING REMARKS**

The carbohydrate of the HIV and SIV Env functions in the proper folding and pro-

cessing of a fusion-competent Env spike, functions to shield the virus from effective antibody-mediated neutralization of infection, functions to bind host lectins to enhance the infection of permissive cells, and functions to trigger signaling pathways resulting in both the productive infection of dendritic cells and the release of immune modulatory cytokines from dendritic cells. The newly highlighted differences in the composition of N-linked and O-linked carbohydrate on HIV-1 gp120 versus SIVsm gp120 may result in these lentiviruses engaging differentially with host lectins. The intriguing question arises whether these differences in carbohydrate composition may modulate the interaction of virus with the host such that humans infected with HIV-1 progress to disease while the majority of naturally infected sooty mangabeys resist generalized lymphoid activation and disease progression despite high levels of SIV replication. Determining whether carbohydrate indeed plays a role in mediating the activated state of the immune response to HIV in humans, and if so, the mechanism behind this activation, will be critical in the development of therapeutics directed toward the carbohydrate of Env.

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